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From pharmacology to physiology: endocrine functions of μ-opioid receptor networks

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Abstract

The steady rise in opioid users and abusers has uncovered multiple detrimental health consequences of perturbed opioid receptor signaling, thereby creating the need to better understand the biology of these systems. Among endogenous opioid networks, μ-receptors have received special attention due to their unprecedented biological complexity and broad implications in homeostatic functions. Here, we review the origin, molecular biology and physiology of endogenous opioids with a special focus on μ-opioid receptor networks within the endocrine system. Moreover, we summarize the current evidence supporting an involvement of the latter in regulating distinct endocrine functions. Finally, we combine these insights to present an integrated perspective on μ-opioid receptor biology and provide an outlook on future studies and unresolved questions in this field.

Keywords

opioids; oprm1; μ-receptors; beta endorphin; hormones; evolution; GPCR

1. Medical use of opioids: an unusual story

The terms “opiates” and “opioids” are often used synonymously but in fact refer to distinct molecular classes: the former comprises natural occurring alkaloids derived from ***Papaver***

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Conflicts of interest

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somniferum (see Glossary) such as morphine, codeine or thebaine, whereas the latter reflects an umbrella term further including peptidergic, synthetic as well as semisynthetic opioid receptor ligands (Figure 1) [1]. In contrast to many other drugs used in modern medicine, the pharmacological implementation of opiates did not arise as a consequence from elucidating the physiology of the underlying system. Instead, opiates have been used for thousands of years, while the molecular basis for their action was only discovered in the late 20th century. Simultaneously, the ongoing opioid crisis (Box 1) has raised awareness for the multiple detrimental health consequences related to perturbed opioid signaling, thereby creating an urgent need to better understand both the physiology as well as pathology of these systems. Among unfavorable opioid-induced side effects, the endocrine system is particularly severely affected by these substances [2], suggesting that opioid receptors hold critical functions in endocrine homeostasis *per se*. While the link between a biological system best-known for its involvement in nociception and endocrine hormones may not be intuitive, we herein relay evolutionary principles such as **life history theory** to opioid biology, thereby providing a possible explanation for the broad involvement of these networks in whole-body homeostasis. This theoretical framework may aid in better understanding the trade-offs arising from opioid receptor activation as well as generating novel perspectives on diseases that could be amenable to manipulation of this system.

2. Biology of endogenous opioids

In the middle of the 20th century, researchers proposed the existence of specific receptors facilitating the analgesic effects of morphine and other opioids, simultaneously raising the question which endogenous ligands might potentially interact with these structures [13]. The discovery of **naloxone**-reversible analgesia arising from electrical stimulation of certain brain regions corroborated the assumed existence of endogenous opioid ligands acting through receptors, which are shared with exogenous opiates such as morphine [14]. Shortly thereafter, endogenous opioid peptides were discovered, followed by the cloning of the three main opioid receptor classes, namely μ-(MOR), κ-(KOR) and δ-(DOR) opioid receptors [15]. The nomenclature of these proteins was based on the first activating ligands to be identified (mu for morphine, kappa for ketocyclazocin) or the initial tissue of discovery (delta for Vas deferens) [16]. Later, the existence of a fourth receptor class coined nociception/orphanin FQ receptor (encoded by OPRL1; opioid-receptor like 1) was confirmed, although the latter differs from other opioid receptors in exhibiting poor binding affinity for cognate opioid peptides [17].

Similar to the three main opioid receptor classes, endogenous opioid peptides are generated from three precursors proteins: preopiomelanocortin, preprodynorphin and preproenkephalin, encoded by POMC, PDYN and PENK, respectively [18, 19]. These precursor proteins undergo post-translational processing (e.g. cleavage by proprotein convertases) to generate a variety of hormones and mature opioid peptides [20]. The latter share a consensus amino acid pentasequence known as the “opioid motif” (Tyr-Gly-Gly-Phe-Met/Leu) [21] (Figure 2). The existence of an additional (fourth) class of endogenous opioid ligands termed endomorphins has also been proposed [22]. Yet, the encoding gene remains unidentified, thus leaving the possibility that these peptides are either exclusively generated via post-translation mechanisms or may not exist after all [23]. Of note, the

characteristics of opioid biology with multiple ligands in the presence of relatively few receptors (convergence) stands in sharp contrast to most other mediators in the CNS, where few ligands typically bind to a plethora of receptors (divergence). Additionally, many functions are shared between different opioid receptors, implying that sufficient engagement of opioid-induced biological programs might be of high priority to organisms (see below).

The long-held belief of distinct receptor selectivity of endogenous opioids has recently been challenged by studies demonstrating wide and potent interactions between different endogenous opioids and opioid receptor classes. Nonetheless, all peptides exhibit varying affinities and potencies to activate individual opioid receptors with the POMC-derivative β-endorphin representing the most potent endogenous agonist for μ-receptors [24]. Given that most desired (analgesia, sedation, anxiolysis, cough suppression), as well as undesired (constipation, respiratory depression, addiction, tolerance, nausea, endocrine perturbations) effects in opioid pharmacology are facilitated by μ-receptors, these signaling networks warrant special attention.

3. OPRM1: from genetics to signaling

G-protein coupled receptors (GPCRs) constitute the largest group of cell membrane proteins expressed across human tissue. GPCRs are evolutionarily related and share conserved genetic as well as structural patterns [25]. As part of the latter family, μ-opioid receptors typically exhibit 7 transmembrane (TM) domains. Together with an intracellular C-terminal tip, these domains are involved into the coupling to small G-proteins and thus, intracellular signaling responses. Transmembrane domains also constitute the ligand binding pocket, whereas N-terminal regions modulate the interaction between ligands and the receptor (Box 2) [26]. In humans and mice, μ-receptors are encoded by a single gene (*OPRM1* and *Oprm1*) consisting of at least 20 and 26 exons, respectively (Figure 3) [27]. Of note, μ-opioid receptors share 94% sequence homology at the protein level between the two species [28]. Phylogenetic studies have mapped the appearance of the quartet of opioid receptors to the origin of jawed vertebrates early in vertebrate evolution approximately 450 million years ago, whereas opioid-like systems have been described in even more primitive invertebrate species (see below) [29].

Over the past two decades, several scientific discoveries have reshaped our understanding of μ-receptor physiology by revealing much greater complexity of these networks than once anticipated. OPRM1 pre-mRNA undergoes extensive **alternative splicing** to generate a plethora of receptor variants or isoforms with distinct biochemical properties. These can be divided into three main classes: 1.) 7-transmembrane C-terminal variants generated by 3’ splicing, all of which contain a consensus sequence built from exons 1, 2 and 3 but exhibit varying intracellular C-terminal amino acid tails; 2.) 6TM variants generated by 5’ splicing that replace exon 1 with exon 11, thereby yielding N-terminally truncated receptors; and 3.) single TM variants, generated by exon skipping or insertion, presumably acting as molecular chaperons for other opioid receptors [27, 30]. Of note, most clinically used μ-receptor agonists require 7TM variants to elicit analgesic effects as demonstrated by the absence of morphine-induced analgesia in Exon 1 knock-out animals devoid of these variants [31]. On the other hand, 6TM μ-receptor variants are responsible for the analgesic effects of a novel

class of opioids such as 3-iodobenzoyl-6β-naltrexamide (IBNtxA), a naltrexone-derivative producing potent analgesia with less side-effects [32–34]. To date, it remains unclear if endogenous opioid peptides can interact with 6TM μ-receptor variants [35]. Of note, overexpression of 6TM variants alone in cell lines *in vitro* did not confer binding of any available radio-labeled opioid ligand [36], raising questions regarding the molecular mechanisms underlying the functions of 6TM variants.

Although the existence of multiple μ-receptor variants has been recognized for years, an appreciation of potential interactions between individual isoforms at the cellular and/or tissue level has only recently emerged. By integrating human RNA sequencing data sets, GPRC sequences and structures as well as proteomics, genetic approaches and pharmacological *in vitro* experiments, Marti-Solano *et al.* revealed conserved patterns across GPRC classes producing similar functional consequences [41]. More specifically, this study demonstrated that N-terminal modifications (resulting from 5' alternative splicing) of such receptors typically alter ligand binding or efficacy, whereas C-terminal changes impact G-protein coupling, signaling and trafficking, which is in line with μ-opioid receptor physiology as outlined above. Most importantly, these authors reported that the combinatorial expression of different isoforms of the same receptor gene has critical consequences for net signaling outcomes (Figure 4). For example, co-expression of the canonical and alternative isoforms of cannabinoid receptor 1 (CNR1) had pronounced impact on intracellular cAMP levels upon **forskolin** exposure.

Taken together, the combinatorial expression of different GPCR isoforms diversifies the cellular response to a given ligand. Hence, predicting physiological and/or pharmacological effects of receptor ligands requires a more holistic approach that has thus far received little attention, which also holds true for μ-opioid receptors.

4. Endocrine functions of μ-opioid receptor networks

Besides the CNS, the highest expression of μ-opioid receptors has been described in endocrine organs such as the testis or the adrenal gland (see human protein atlas, GTEx dataset or [42]) with an accumulating body of evidence supporting the existence of peripheral opioid networks functioning locally within such tissues. Taken together with the observation that chronic opioid use has been linked to a variety of endocrine diseases such as hypogonadism and infertility, osteoporosis, adrenal insufficiency, as well as diabetes, an involvement of μ-opioid receptor networks in endocrine homeostasis appears likely [2, 43]. Indeed, a large body of preclinical and clinical evidence has implicated μ-receptors and their ligands in regulating endocrine systems. Although the implementation of the **Cre-LoxP-system** has allowed scientists to explore organ-specific functions of selected genes and both global as well as conditional μ-opioid receptor knock-out mice have been generated [44, 45], surprisingly little effort has been invested into exploring the causal involvement of these networks in endocrine health and disease. The following sections will summarize the currently available evidence for such functions of μ-receptors with a special focus on peripheral effects on endocrine tissues.

4.1. Central and peripheral effects on reproduction

In both men and women, opioid receptor activation yields a reduction of gonadotropin releasing hormone (GnRH) release paralleled by a decrease in circulating sex hormone levels [46]. Indeed, **hypogonadism** is commonly observed among opioid users with varying prevalence depending on the substance used, duration of the treatment as well as the route of administration. In male patients receiving opioid therapy for non-cancer pain, 20–85% exhibit hypogonadism depending on the testosterone threshold used to define the condition [47]. As for most data reported in such patient collectives, these findings are likely blurred by confounders such as pain, concomitant medications or underlying diseases. Nevertheless, a plethora of experimental evidence supports a direct involvement of opioid receptors in regulating reproductive functions.

Administration of naloxone to male rats provoked an increase in luteinizing hormone (LH) levels, which was reversed by co-administration of morphine [48]. Similarly, chronic treatment of male rabbits with naloxone for 14 days resulted in increased circulating LH and testosterone levels [49]. Likewise, opioid receptor blockade in men evoked increased LH pulse frequency and enhanced sex hormone secretion [50, 51]. Similar observations have been made in females, where acute morphine administration yielded a suppression of LH levels [52]. Together, these findings led researchers to hypothesize that opioids might be involved in facilitating the negative feedback inhibition of GnRH release by sex hormones. Indeed, circulating β-endorphin levels rise during the follicular phase of the menstrual cycle in healthy women and increase even further in the luteal phase, thus paralleling the peak of progesterone-mediated feedback inhibition of GnRH release [53]. The crucial involvement of β-endorphin in this process is further highlighted by studies performed in rhesus monkeys [54]. Here, ovarectomized animals were supplemented with either estradiol or estradiol/progesterone and β-endorphin levels were measured in hypophyseal portal blood. Whereas the former treatment had only mild effects on β-endorphin levels, the latter provoked a strong increase, reminiscent of circulating β-endorphin kinetics found in humans as noted above. Consistently, the suppression of LH pulse frequency induced by continuous dihydrotestosterone infusion was reversed by naloxone administration in healthy women, further supporting the concept that opioid receptors play a role in sex hormone-mediated feedback inhibition [55].

Of note, most of these studies do not allow to preclude the possibility that opioid-receptors other than μ-receptors might be involved in facilitating the reported effects. However, the preferential binding of both exogenous (morphine, naloxone), as well as endogenous opioid receptor ligands (β-endorphin) investigated in these studies [56], corroborates the hypothesis that these receptors are crucially involved in governing hypothalamic GnRH release. Of note, β-endorphin-deficient mice (devoid of the principle endogenous μ-receptor ligand) apparently breed normally [57], suggesting that functional redundancy within the endogenous opioid system allows for compensatory μ-receptor activation or engagement of these receptors is dispensable to reproduction after all. Conversely, an increased endogenous opioid tone apparently perturbs fertility as noted above.

More recently, peripheral opioid receptor networks have been implicated in contributing to the regulation of reproductive functions. In women undergoing in vitro fertilization (IVF), β-endorphin concentrations in follicular fluid predicted the number of retrieved metaphase II oocytes, thus pointing towards an involvement of endogenous opioids and μ-receptors in oocyte maturation [58]. Indeed, the presence of MOR on oocytes has been confirmed [59]. Subsequent studies revealed that follicular fluid β-endorphin content associates with pregnancy outcomes and live birth rates in women with **polycystic ovary syndrome** and diminished ovarian reserve undergoing IVF, further underpinning a potential role of opioid peptides in oocyte maturation [60]. Additionally, β-endorphin levels in follicular fluid of healthy young women were found to be 10–40 times higher than those in plasma, suggesting local synthesis and/or clearance mechanisms [61]. Of note, opioid receptors are also expressed by male germ cells [62]. Similar to reports in women, β-endorphin concentrations in seminal plasma exceeded those in systemic plasma by a factor >10 [63]. Intriguingly, *in vitro* exposure to morphine increased the number of immotile sperm cells [64], mirroring the clinical picture of impaired semen quality in heroin abusers [65]. Finally, β-endorphin and met-enkephalin have been detected in the uterine fluid of women with subsequent studies revealing local POMC expression in primary endometrial tissue [66, 67]. Consistently, endometrial OPRM1 mRNA and protein expression reach a maximum at the time of ovulation, further supporting a physiological function of μ-receptor networks in the uterine cavity [68]. It is worth mentioning that other opioid receptor classes (i.e. DOR and KOR) have been detected in peripheral reproductive tissues as well [59]. In view of the rather high binding affinity of β-endorphin to κ- and δ- receptors (see above), an involvement of these receptors in facilitating some of the peptide's peripheral effects is likely.

In summary, preclinical and clinical evidence implicates a critical involvement of μ-opioid receptors in regulating the hypothalamic pituitary gonadal axis, effects which are mainly of inhibitory nature. On the other hand, the existence of peripheral opioid networks in reproductive tissues has been discovered only recently and remains to be further explored.

4.2. Regulation of glucose homeostasis

In contrast to recent discoveries of opioid receptor networks in the gonads, peripheral effects of opioid peptides on the endocrine pancreas have long been appreciated. Almost three decades ago, β-endorphin was found to inhibit insulin release from isolated pancreatic beta cells [69]. Likewise, β-endorphin infusion strongly decreased pancreatic insulin secretion in healthy volunteers, while stimulating glucagon release, effects which were found to be cAMP-dependent [70]. In contrast, ambiguous results were reported by other authors with differential effects of β-endorphin on insulin kinetics depending on glucose concentrations and whole-body metabolism [71–73].

The arguably most compelling evidence for an involvement of μ-opioid receptor networks in modulating pancreatic beta cell function stems from global μ-receptor knock-out mice (*MOR−/−*) [74], which were generated by targeting exon 1 of the *Oprm1* locus. This approach renders mice devoid of all 7TM variants, while leaving 6TM μ-receptor variants intact. On a regular chow diet, these animals exhibited exaggerated weight gain compared to wildtype littermates, which was explained by an apparent increase in adipose tissue fat mass.

Intriguingly, these effects were observed as early as 5 days after birth. Phenotypic characterization of these mice revealed enhanced glucose tolerance characterized by pronounced hyperinsulinemia. Most interestingly, *MOR−/−* animals displayed a prominently increased pancreas weight with enhanced beta cell mass and heightened insulin content. Consistently, pancreatic beta cells isolated from knockout mice secreted more insulin upon **tolbumatide** exposure than cells derived from wildtype controls. Taken together, these data suggest that 1.) μ-receptors hold crucial inhibitory functions in modulating insulin release 2.) pancreatic beta cell proliferation is partly governed by μ-receptors and 3.) a loss of opioid-tone (activity) results in an anabolic state characterized by hyperinsulinemia and weight gain. In view of the global nature of the receptor knockout and the lack of information on food intake provided by the authors, contributions of central effects of μ-receptors to the phenotype cannot be excluded. Indeed, μ-receptors also modulate insulin release via mechanisms in the CNS. Intracerebroventricular administration of the μ-agonist DAMGO blunted glucose-stimulated insulin release in mice in an alpha2-adrenoreceptor dependent fashion, suggesting additional indirect effects via the sympathetic nervous system [75]. On the other hand, an independent μ-opioid receptor knock-out strain did not exhibit overt metabolic abnormalities when fed ***ad libitum.*** Yet, these mice displayed resistance against high fat diet-induced obesity [76], which stands in sharp contrast to findings from other *MOR−/−* mice reported above. Importantly, this strain was generated by targeting exons 2 and 3 of the *Oprm1* locus, thus yielding a predicted loss of all 7TM and 6TM, while not affecting single TM μ-receptor variants [77]. Taken together, 6TM and 7TM μ-receptor variants may hold distinct (and even opposing) physiological functions, which highlights the necessity to consider the genetic complexity of μ-receptors when interpreting experimental data.

Finally, endogenous opioids are heavily involved in reward circuits, regulating food intake and hedonistic behavior with μ-receptor agonists generally promoting feeding irrespective of satiety, both in vertebrate, as well as invertebrate species [78, 79]. Consistently, opioid receptor blockade by a naltrexone-bupropion sustained release formulation elicited weight loss in obese individuals with type II diabetes, which was paralleled by improvements in glycemic control [80]. Similar observations have been made in overweight individuals with polycystic ovary syndrome [81]. Of note, the pharmacological utility of naltrexone is compromised by its side effects with a large percentage of patients experiencing nausea and/or vomiting [80].

4.3. Modulation of the stress response

Given the apparent homeostatic regulation of glucose metabolism by μ-receptors, one may wonder whether these networks are also involved in other immediate responses requiring glycemic adaptations such as the stress (fight and flight) response. Indeed, significant expression of μ-receptors has been reported in the adrenal gland ([82] and GTEx dataset). However, which functions these receptors fulfill in this tissue remains largely unclear with some *in vitro* studies reporting inhibitory, while others demonstrating stimulatory effects on catecholamine and glucocorticoid secretion, respectively [83–85]. Yet, in patients with hypothalamic-pituitary disconnection, naloxone administration increased cortisol, but not andrenocorticotropic hormone (ACTH) levels, supporting the idea of direct effects of

opioids on the adrenal glands [86]. Likewise, β-endorphin infusion suppressed circulating catecholamine levels in hypertensive and healthy subjects, respectively, further supporting inhibitory actions of μ-receptors on adrenal gland responses [87].

Centrally, μ-receptors generally appear to inhibit hypothalamic-pituitary-adrenal (HPA) axis activity. Male heroin addicts on methadone maintenance therapy exhibited reduced circulating ACTH and cortisol levels both basally, as well as after **metyrapone** administration [88]. Consistently, β-endorphin infusion reduced circulating cortisol levels in healthy volunteers [89]. Moreover, a single nucleotide polymorphism (SNP) in the *OPRM1* gene (A118G) proposed to confer enhanced ligand binding to the receptor has been linked to blunted ACTH responses in humans undergoing metyrapone testing [90]. In line with these observations, long-term opioid treatment may evoke hypoadrenalism [91], further underpinning the inhibitory effects of opioid receptor activation on HPA axis activity.

Of note, SNPs within the OPRM1 locus eliciting functional consequences reminiscent of those found in humans have also been described in non-human primates [92, 93], demonstrating how common evolutionary pressures may have produced similar phenotypes across species. Furthermore, these findings could imply a shared genetic response to environmental challenges mediated by opioid receptor networks [92].

Finally, chronic opioid use has also been associated with the development of another common endocrine disease, namely osteoporosis. However, the underlying mechanisms remain incompletely understood (Box 3).

5. An integrated perspective on μ-opioid receptor networks

The evolutionary advantage conferred by the implementation of endogenous opioid networks is highlighted by several observations. First, opioid-like systems are not confined to higher developed organisms but also found in more primitive invertebrate species such as *Molluscs, Annelids* or *Arthropods* [99]. Second, the structure of opioid receptors is highly conserved across vertebrate species despite originating early in their evolution (see above). Third, opioid receptor networks are involved in the regulation of central physiological functions such as reproduction or feeding behavior and fourth, the high homology between different opioid receptor subclasses as well as their redundant activation by different endogenous opioids indicates that ensuring sufficient engagement of these networks is of high priority to organisms.

From an evolutionary perspective, reproductive fitness reflects the main driver of natural selection. Thus, a genetic repertoire conferring optimal adaptation to a given environment (resulting in maximized reproductive fitness) will be selected for. Consistently, traits mediating such adaptive responses are of particular importance to organisms [100].

In *Caenorhabiditis elegans*, endogenously expressed nlp-24 (neuropeptide-like protein 24)-derived peptides were found to exert agonistic activity at an opioid-like receptor (nrp-17), which could also be activated by prototypical pharmacological opioid receptor agonists such as morphine [101]. Engagement of this system promoted locomotion and feeding behavior in starved animals, both of which aid in nutrient uptake under challenging conditions,

suggesting that opioids mediate survival responses. In vertebrates, the versatile effects arising from μ-opioid receptor activation might seem unrelated to such functions. Yet, the net organismal outcome elicited by these programs may be summarized as a state of energy preservation, also referred to as maintenance. The latter reflects an orchestrated response to constraints imposed by unfavorable environmental conditions (e.g. nutrient scarcity). Collectively, maintenance programs are characterized by an accentuation of catabolic pathways at the expense of anabolism (i.e. growth and reproduction), corresponding to a prioritization of resource allocation through trade-offs [102].

Indeed, prolonged μ-opioid receptor engagement, either invoked exo- or endogenously, yields a cessation of multiple programs that are energetically costly: reproduction (inhibition of GnRH release) [52], macromolecule synthesis und nutrient storage (reduction of insulin secretion) [74], tissue renewal (decreased bone formation) [94], cardio-respiration (attenuated cardiac function, hypotension and respiratory depression) [1], digestion (decreased gastrointestinal motility) [34], fight and flight response (reduced HPA axis activity) [91] as well as the pro-inflammatory response (immunosuppression) [103]. These adaptations are crucial in face of challenging environmental conditions due to the inherently finite nature of organismal resources.

The most impressive examples for these annotations are hibernating animals, who temporarily suppress a broad range of metabolic functions (including most anabolic programs) in order to survive in unfavorable environments [104]. Intriguingly, intracerebroventricular administration of naloxone to hibernating Syrian hamsters provoked an arousal response, suggesting that endogenous opioids might be involved in facilitating maintenance programs [105]. Indeed, subsequent studies revealed an increase in β-endorphin immunoreactivity in the arcuate nucleus of these animals after hibernation onset and anti-β-endorphin antibodies elicited arousal, reminiscent of the effects of naloxone noted above. Conversely, intracerebral administration of the selective μ-receptor agonist DAMGO invoked a sharp decrease in body temperature, a characteristic adaptation occurring in hibernating species when engaging such programs [106].

Taken together, these findings suggest a role of central μ-receptors and their principle endogenous ligand β-endorphin in facilitating adaptations to challenging environments, which are crucial to survive. Pertinent to this, the strongest triggers for endogenous β-endorphin release thus far reported comprise painful stimuli, exhausting exercise, fasting or stressful tasks, all of which indicate challenging environments and are similarly found in invertebrate species [99, 107–109]. In mammals, the common molecular origin of ACTH and β-endorphin (i.e. POMC) should be considered when interpreting such findings since elevated β-endorphin levels under these conditions could partly arise from increased HPA axis activity.

Although humans do not hibernate, the adaptations occurring in response to environmental challenges are similar to other mammals: prolonged stress, excessive exercise and/or caloric mismatch yield hypogonadism (as exemplified by the clinical entity of hypothalamic amenorrhea) as does cold exposure, infection or any other evolutionary relevant environmental stressor [110–113]. Similarly, bone formation is typically decreased in such

individuals, thus conferring a risk for the development of osteoporosis and fractures [113]. Pertinent to an involvement of central opioid receptors in facilitating such responses in humans, patients with hypothalamic amenorrhea have successfully been treated with opioid antagonists (i.e. naltrexone) to restore gonadal-axis activity and even induce pregnancy [114].

Thus, temporal increases in endogenous opioid tone likely confer a significant survival advantage to organisms through facilitating a variety of systemic adaptations, which are conserved across species and tailored to demands arising in challenging environments. On the other hand, a persistently increased opioid tone may eventually become maladaptive and contribute to disease since the engagement of this system occurs at the expense of certain other aspects of physiology (trade-off) (Figure 5). The relative contributions of central vs. peripheral μ-opioid receptor networks to these phenomena remain unknown.

6. Concluding remarks and future perspectives

The ongoing opioid crisis has reignited the scientific interest in opioids and specifically, μ-receptors. Although a considerable body of evidence supports an involvement of these signaling networks in endocrine homeostasis, much of our knowledge on μ-receptors is derived from older studies that were unable to address critical questions due to the lack of adequate molecular techniques in former times, thereby yielding an underestimation of the respective biological complexity. Indeed, recent advances in μ-opioid receptor research have highlighted the necessity to consider differential effects elicited by truncated and full-length receptor variants, although the physiological (including endocrine) functions of the former remain poorly understood.

The theoretical framework presented in this manuscript suggests that manipulation of opioid receptor signaling may reflect a widespread, conserved organismal effector mechanism in response to constraints imposed by challenging environments. The resulting trade-offs arise from the prioritization of organismal resource allocation and may aid in understanding many of the unfavorable health consequences elicited by prolonged opioid therapy. Vice versa, opioid receptors could perhaps be pharmacologically targeted in a much broader context than presently appreciated. However, this transition needs to be preceded by a significant body of research to unravel the basic homeostatic functions of endogenous opioid receptor networks beyond pain perception, both in- and outside the CNS.

Now, it is up to scientists to harness the available tools to elucidate the plethora of outstanding questions, all of which will aid in better understanding the intricate complexity of these networks.

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Glossary

Papaver somniferum
also known as „opium poppy"; species of flowering plant; contains several alkaloids, some of which potently bind to opioid receptors (collectively referred to as opiates) including morphine and codeine

life history theory
theoretical framework seeking to explain how organisms allocate their resources into three main biological programs (growth, reproduction, maintenance) in order to maximize reproductive success; investment strategy is dictated by the quality of the environmental conditions encountered

naloxone
synthetic, unselective, competitive opioid antagonist; highest binding affinity for μ-opioid receptors

alternative splicing
regulated process occurring after gene transcription; enables the production of multiple proteins from a single genetic sequence; relies on the creation of different exon combinations in the mature mRNA

DAMGO
[D-Ala^2, *N*-MePhe^4, Gly-ol]-enkephalin; synthetic enkephalin-derivative; exhibits high affinity and specificity for μ-opioid receptors, which contrasts the preferential binding of endogenous enkephalins to δ-opioid receptors

forskolin
geranylgeranyl-pyrophosphate derivative naturally occurring in plants (*Plectranthus barbatus*); experimentally used as a potent adenylate cyclase stimulator; provokes an intracellular cAMP increase

Cre-LoxP-system
site-specific recombinase technology; mostly used to insert or delete specific DNA sequences, thus yielding "knock-in" and "knock-out" phenotypes, respectively; consist of the enzyme Cre (causing recombinase) and loxP sites (locus of x-over, P1), the latter flanking the genetic sequence of interest

hypogonadism
insufficient gonadal function characterized by reduced circulating sex hormone levels and associated clinical symptoms; may be accompanied by infertility

polycystic ovary syndrome
also known as PCOS; common endocrinopathy among women in reproductive age; characterized by biochemical and/or clinical hyperandrogenemia, menstrual cycle abnormalities and polycystic morphology of the ovaries; presence of two out of three listed symptoms justifies a PCOS diagnosis (Rotterdam criteria)

tolbutamide
first-generation potassium channel blocker; yields a depolarization of pancreatic beta cell membranes, thereby provoking opening of voltage-gated calcium channels and subsequent insulin release

ad libitum
“as desired”, animals have free access to food

metyrapone
reversible CYP11B1 (11-beta-hydroxylase) inhibitor; blocks adrenal cortisol synthesis; yields an increase in circulating 11-deoxycortisol, paralleled by enhanced corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) secretion due to a loss of negative feedback inhibition; used in endocrine practice to exclude tertiary adrenal insufficiency/evaluate hypothalamic-pituitary-adrenal (HPA) axis functionality

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Outstanding questions

1. Do endogenous opioids interact with 6TM μ-receptor variants?
2. Does the selective 6TM variant agonist IBNtxA invoke similar endocrine side-effects as “classical” opioids?
3. What is the relative contribution of G-protein vs. β-arrestin-dependent signaling to endocrine side effects elicited by opioid therapy?
4. Do peripheral tissues significantly contribute to circulating β-endorphin levels?
5. Do combinatorial expression profiles of μ-receptors differ between endocrine organs? Does this allow the creation of tissue-specific ligands?
6. Are central and peripheral opioid receptor networks mechanistically interconnected or do they function autonomously?
7. Which factors regulate the production of opioid peptides in the periphery?
8. Is the increase in circulating β-endorphin under stressful conditions blunted in animals with hypophyseal-specific POMC deletion?
9. Can μ-receptor ligands be used *in vitro* to improve oocyte quality?
10. Does the microbiome contribute to shaping the endogenous opioid tone of the host? If so, how is this accomplished?

Highlights

- The endogenous opioid system holds crucial functions in endocrine homeostasis
- Opioid peptides are not only produced in the CNS, but also in peripheral organs such as the gonads
- Alternative splicing mechanisms give rise to a plethora of μ-opioid receptor variants with distinct biochemical properties
- The combinatorial expression of different μ-receptor variants determines signaling responses in a given cell or tissue
- Opioid networks fulfill conserved functions across species
- The net organismal outcome of μ-receptor activation reflects a state of energy preservation (maintenance), which is a key adaptative response of organisms to survive in challenging environments
- Persistent opioid receptor engagement (either elicited exo- or endogenously) occurs at the expense of distinct physiological programs (e.g. reproduction), thus eventually invoking disease

Box 1:

Trajectories of the opioid crisis

In 1980, a one-paragraph letter postulating a low risk for the development of addiction following opioid treatment was published in the *New England Journal of Medicine* [3]. The authors' claim was based on a retrospective data evaluation revealing that few hospitalized patients (4 out of 11882) developed relevant addictive disease subsequent to an opioid prescription. Although no further evidence supporting this hypothesis was presented, the same article has been widely cited (> 600 times) in the scientific literature as evidence that opioid use confers negligible risks for addiction [4].

Today, the United States and Canada (and to a lesser extent, other developed countries of the world) are facing an opioid crisis characterized by increasing numbers of overdose-related deaths from both legally as well as illegally obtained opioids [5–7]. In 2018 alone, 46 802 Americans succumbed to a lethal opioid overdose, corresponding to an average of 128 opioid-related deaths per day [8]. Although the above cited article may not be held responsible for the current crisis, it is indeed representative of the changing perspective on opioids that arose around this time. While these substances were traditionally used to treat acute pain related to injury or terminal illness, an increasing awareness for alleviating chronic pain conditions advocated by both scientific publications as well as the World Health Organization became apparent in the 1980s. The subsequent development and introduction of semisynthetic opioids such as Oxycodone as well as aggressive marketing strategies (most prominently illustrated by the case of OxyContin) further fueled increasing opioid prescriptions across the US [9]. Additionally, studies indicating insufficient pain control in a large percentage of patients with chronic illnesses, as well as novel remuneration structures in the health care system rewarding patient satisfaction (whereby pain control took a great share of the overall score) also contributed to this evolution [9, 10]. Finally, an increasing number of semisynthetic opioids became widely available, thus increasing the accessibility, while lowering the cost of the individual compound. Of note, these developments did not remain unnoticed and prescribed opioid doses leveled off by 2010 in the US [11]. Conversely, the number of deaths related to illicit opioid use continue to rise, suggesting that the distribution and availability of these substances among the population remains high [6]. Taken together, the opioid crisis imposes a substantial socioeconomic burden with an estimated annual cost of almost 80 billion dollars in the US [12].

Box 2:

Intracellular signaling of μ-opioid receptors

MORs belong to the GPCR class A rhodopsin family and typically couple to Gi/o proteins. As such, MORs mainly harness two distinct intracellular signaling pathways, one of which relies on G-protein signaling, the other being facilitated by β-arrestin proteins. Activation of the G-protein dependent branch typically yields a reduction in intracellular cAMP levels through the inhibition of adenylatcyclases with subsequent effects on protein kinases and/or ion channels, ultimately culminating in dampened neuronal excitability and/or cellular responses. On the other hand, ligand binding to μ-receptors may also trigger intracellular phosphorylation events, thereby promoting the recruitment of β-arrestin proteins, which subsequently bind to the receptor, attenuate further G-protein signaling and may foster internalization of the protein. However, β-arrestin binding also redirects the cellular response to alternative pathways such as mitogen activated protein kinase signaling [37]. Overall, β-arrestin-dependent pathways are believed to account for the majority of undesired consequences of long-term opioid use including the development of tolerance as indicated by the absence of the latter in β-arrestin-2 knock-out mice [38]. These observations led researchers to hypothesize that synthesizing “biased” opioid receptor ligands favoring G-protein over β-arrestin-dependent signaling might be sufficient to overcome many obstacles of current opioid pharmacology, a promise that has thus far not been convincingly fulfilled. Crucially, endogenous opioids themselves are biased agonists with different peptides preferentially activating one of the two intracellular signaling branches [24]. Further complexity to the μ-receptor system is added by the fact, that alternatively spliced C-terminal variants differ in their inherent bias for promoting G-protein vs. β-arrestin-dependent signaling [39, 40]. Thus, μ-opioid receptor signaling bias is created at both the ligand, as well as the receptor level.

Box 3:

Opioids and bone health.

Chronic opioid use has been associated with impaired bone quality and an increased fracture risk [94]. Mechanistically, opioid-induced hypogonadism likely plays a prominent role in this pathology, while an enhanced susceptibility to falls secondary to dizziness may also contribute. Additionally, direct effects of opioids on bone have been proposed [95]. Yet, experimental evidence supporting this hypothesis is scarce. A widely cited publication demonstrating morphine-induced inhibition of osteocalcin production used excessively high doses (mM range) that are unlikely to occur neither physiologically, nor pathologically [96]. Moreover, the cell line used to study these effects (MG-63) was derived from sarcoma tissue and poorly resembles primary osteoblast characteristics [97]. Finally, the detection of opioid receptors in bone cells has thus far relied on semi-quantitative techniques and suboptimal primer design with most studies failing to detect relevant expression levels of any opioid receptor subclass in bone [98]. Of note, global dynorphin knock-out mice (*Dyn*−/−) displayed enhanced bone mass, although this effect was found to be mediated via the CNS, rather than peripherally [98].

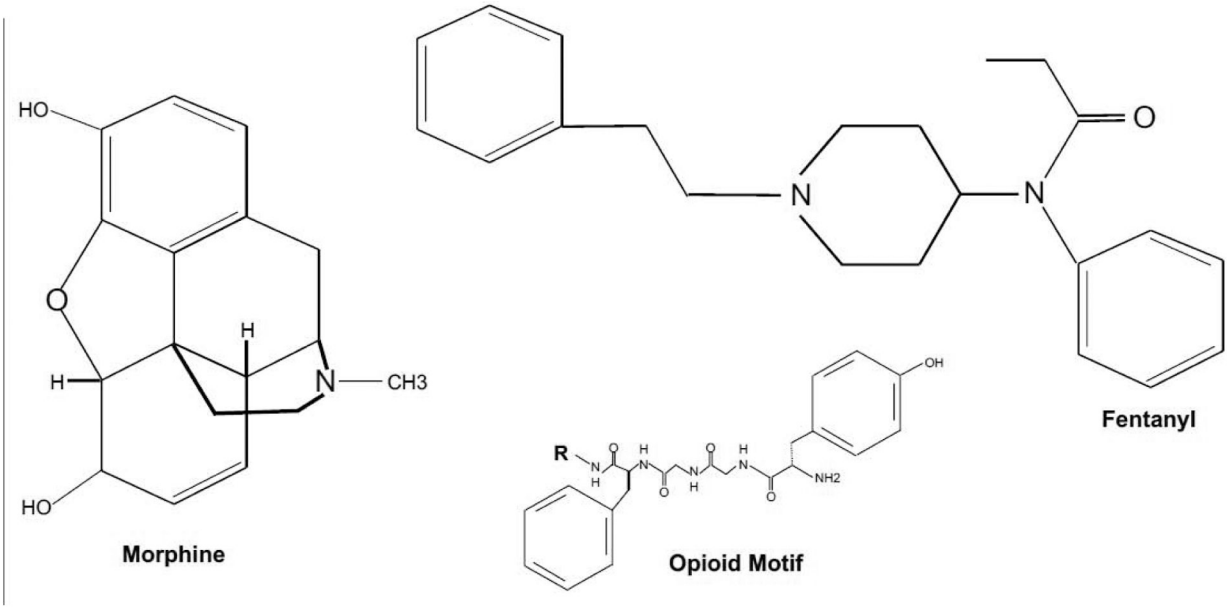

Figure 1: Chemical structure of opioids.

Alkaloids derived from *Papaver Somniferum* including morphine or codeine share a common carbon-scaffold and preferentially bind to μ-opioid receptors. Semi-synthetic opioids such as Fentanyl bind and activate these receptors even more potently. Endogenous opioids all share a core amino acid pentasequence coined the “opioid motif” consisting of Tyr-Gly-Gly-Phe-Met or Leu. Enkephalins are exclusively built from these 5 amino acids, whereas all other opioids peptides exhibit various additional residues. The core opioid tetrasequence with “R” indicating the respective residue (Met/Leu-XXX) is shown above.

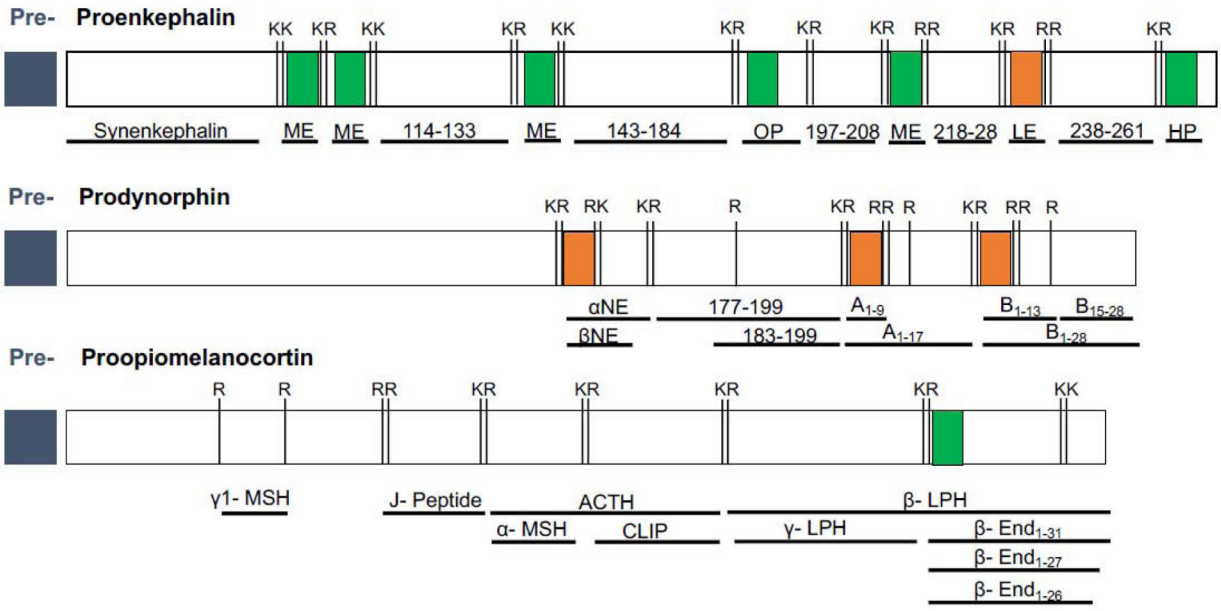

Figure 2: Endogenous opioid peptides are generated from precursor proteins.

Schematic illustration of the three major opioid peptide precursor proteins (conserved between mice and humans with subtle differences; the former shown above): preproenkephalin (PENK), preprodynorphin (PDYN) and preproopiomelanocortin (POMC). Major cleavage sites consisting of Lysine (K) and Arginine (R) residues are indicated. The two core opioid motifs are highlighted in green (Tyr-Gly-Gly-Phe-Met) and orange (Tyr-Gly-Gly-Phe-Leu), respectively. Post-translational processing of each precursor generates a variety of different hormones and peptides, some of which have not been named (referred to as numbers in the figure). Lower case numbers reflect different isoforms of the same peptide that vary in their length and thus, biological activity. Note that cleavage of POMC also generates non-opioid mediators such as ACTH or MSH (Adapted from [42]).

ME= Met-enkephalin, OP=octapeptide, LE= Leu-enkephalin, HP=heptapeptide, αNE= alpha neoendorphin, βNE= beta neoendorphin, A_{x-y}= dynorphin A variants, B_{x-y}= dynorphin B variants, γ1-/α- MSH= gamma1/alpha melanocyte stimulating hormone, J-Peptide= joining peptide, CLIP= corticotropin-like intermediate lobe peptide; β-/γ-LPH = beta/gamma Lipotropin; β- End_{x-y}=beta endorphin variants

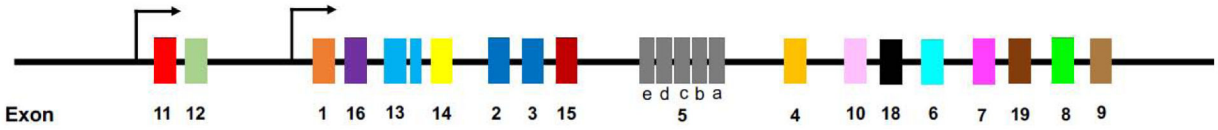

Figure 3: The genetic complexity of μ-receptors.

Although only a single μ-receptor encoding gene exists in humans and rodents (the latter shown above), alternative splicing of the OPRM1 pre-mRNA gives rise to a plethora of receptor variants that exhibit distinct biochemical properties. All 7TM variants contain a consensus sequence built from exons 1, 2 and 3, whereas 6 TM variants replace exon 1 with exon 11. Note that the exon nomenclature does not reflect their chromosomal locus but rather their timepoint of discovery.

$$\sum_{i=1}^{n} (a_i \times b_i) = (a_1 \times b_1) + (a_2 \times b_2) + (a_3 \times b_3) \ \ldots (a_n \times b_n)$$

$\sum_{i=1}^{n}$	Net receptor signaling outcome (weighted sum of individual isoform effects)
n	Total number of receptor variants present in a cell/tissue
$a_i \ldots a_n$	Signaling effect of individual isoforms (can be positive or negative)
$b_i \ldots b_n$	Weighting factor of the respective receptor variant; relative contribution to overall outcome; Influenced by expression and ligand binding affinity

Figure 4: Expression profiles of GPCR variants determine net signaling outcomes.

Simplified mathematical modelling of signaling responses arising from the combinatorial expression of different GPCR variants in a given cell or tissue.

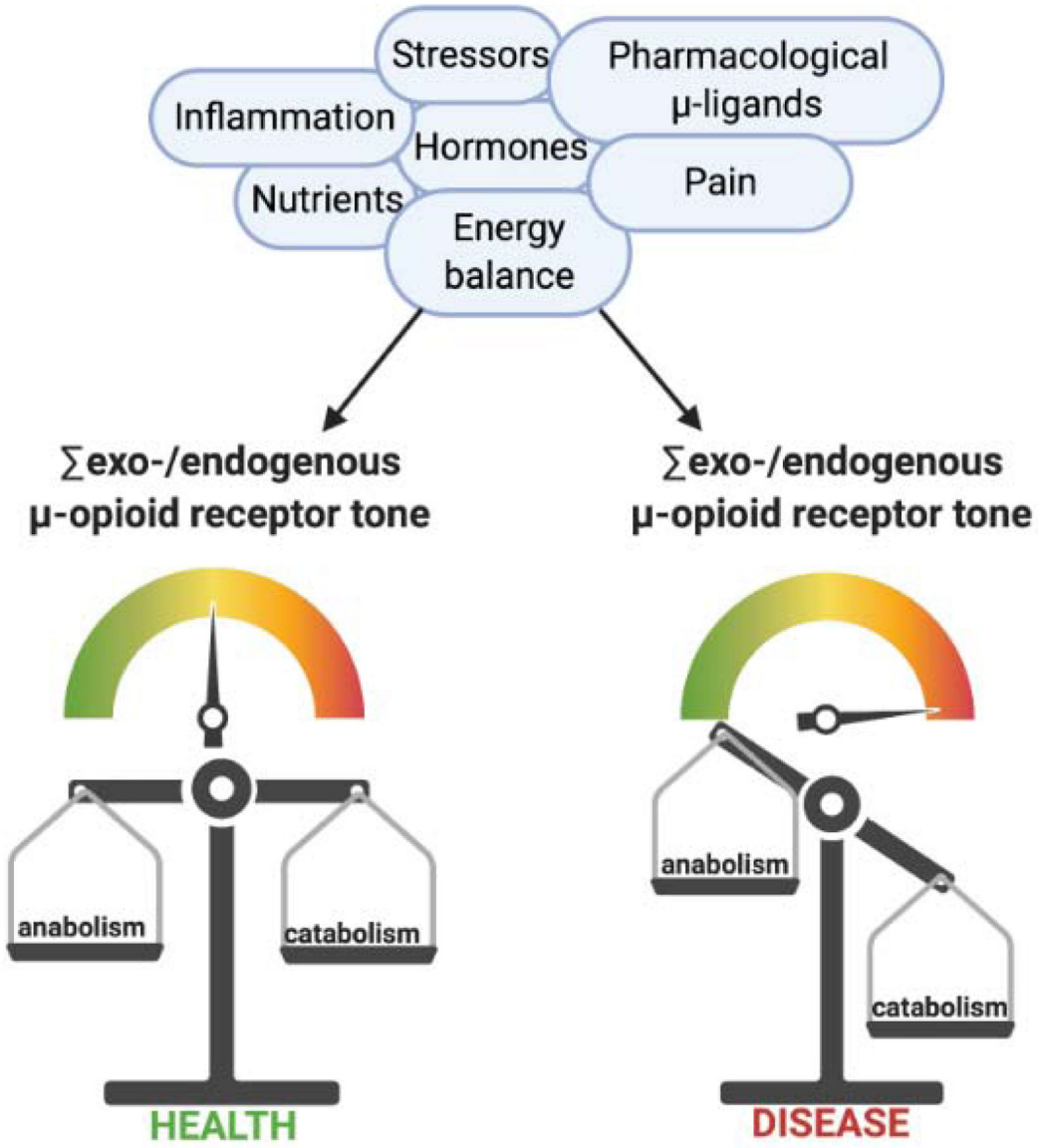

Figure 5: Perturbed μ-opioid receptor activity contributes to disease.

Engagement of μ-opioid receptors (sum activity) emanates from both exogenous (pharmacological), as well as endogenous sources (endogenous opioid peptides), the latter being shaped by a plethora of factors as exemplified above. Overall, biological responses prompted by μ-receptor activation occur at the expense of energetically costly (anabolic) programs such as reproduction, the immune response or bone formation. While such changes may be temporally beneficial in the face of challenging environments, their persistence (e.g. due to long-term opioid treatment) culminates in disease.